MÖSSBAUER SPECTROSCOPY OF IRON-CONTAINING NANOPARTICLES FOR BIOLOGICAL APPLICATIONS

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1. Introduction

Mössbauer spectroscopy is a versatile technique that can be used to provide information in many areas of science such as Physics, Chemistry, Biology and Metallurgy. It can give very precise information about the chemical, structural, magnetic, time- and temperature dependent properties of a material. Key to the success of the technique is the discovery of recoilless gamma ray emission and absorption, now referred to as the Mössbauer Effect, after its discoverer Rudolph Mössbauer, who first observed the effect in 1957 and received the Nobel Prize in Physics in 1961 for his work [1].

We were studied of materials for biomedicine. First is determination of biological tissues, second one is characterization of nanoparticles for hyperthermia.

Free iron is toxic to for the biological tissues-cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species via the Fenton Reaction [2]. In humans, it acts as a buffer against iron deficiency and iron overload [3]. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body; hence serum ferritin is used as a diagnostic test for iron deficiency anemia [4].

The next powder sample for biomedicine are the magnetite-derived ferrites which are important magnetic materials used as electronic elements or permanent magnets and nowadays also in biomedicine in the form of nanoparticles for magnetic fluid hyperthermia, magnetic resonance imaging, separation of cells and drug targeting [5-6]. As far as the magnetic fluid hyperthermia is concerned, the destruction of the tumorous tissue is achieved by a heating effect of incorporated nanoparticles exposed to alternating magnetic field. There are two crucial needs for their application, namely the high specific power losses leading to high heating efficiency in order to minimize the injected dose and the elimination of local overheating by a self-controlled heating mechanism. The $Co_{1-x}Zn_xFe_2O_4$ systems are ferrimagnets with Curie temperatures (T_C) fast decreasing with increasing Zn content [7]. The value reported for pure $CoFe_2O_4$ is T_C about of 520 ^{O}C for bulk and T_C about of 460 ^{O}C for the 12 nm particles. The heating efficiency can be optimized by adjustment of magnetization and coercivity, in which the zinc doping proved effective [7].

2. Experimental details

The samples from a human brain, human and horse spleen were extracted post mortem at the Department of Pathological Anatomy, Comenius University in Bratislava, in accordance with the Helsinki Declaration. The samples of human brain were extracted from the region Globus Pallidus which is a part of Basal Ganglia. Fresh, soft tissues were dried in a vacuum (lyophilized) and the resulting samples were obtained in a form of powder.

Synthesis of the Co–Zn ferrites in nanocrystalline form was carried out by the coprecipitation method followed by a temperature treatment. The analyzed materials, Co(II), Zn(II) and Fe(III) nitrates (purity grade p.a. (for analysis)) were dissolved in water and mixed together in appropriate proportions of cations, corresponding to the chemical formula $Co_{1-x}Zn_xFe_2O_4$ (x = 0.5, 0.6 and 0.7). All preparation details are described in [8].

Mössbauer spectroscopy has been chosen as a main tool of structural characterization of all type of the samples. Mössbauer spectra of the sample were acquired in transmission mode using a conventional constant acceleration-type spectrometer equipped with a $^{57}Co(Rh)$ source embedded in a rhodium matrix at room temperature and for selected samples also at the temperature of 4.2 K using liquid helium bath cryostat. Calibration of the velocity scale was performed with a thin (12.5 μm) α-Fe foil and isomer shifts are given with respect to its room temperature Mössbauer spectrum. Mössbauer spectra obtained at room temperature from all investigated samples exhibit several different components depending of the type of iron-containing materials. At room and low temperatures, the samples were measured in range ± 12 mm/s. Mössbauer spectra of biological tissues measured also in a narrow velocity range ± 4 mm/s to allow better resolution of the spectral lines. All spectra were evaluated using the program CONFIT [8].

Morphology of samples was revealed by SEM (Scanning Electron Microscopy). The scanning electron microscopes Tescan with BSE detector were employed to visualise contrast between areas with different chemical compositions. Diffraction patterns were collected in reflection mode with a PANalytical X´Pert PRO diffractometer equipped with a conventional X-ray tube (Co Kα radiation, 40 kV, 30 mA, line focus) and a multichannel detector X'Celerator with an anti-scatter shield. X-ray patterns were measured in the range of 20 to 150° of 2 Θ with step of 0.0167° and 700 s per step.

3. Results and discussions

Standard powder X-ray diffraction experiment was performed aiming at the identification of possible iron structures (hematite, maghemite, magnetite, ferrihydrite) in the spleen and brain tissues.

Mössbauer spectra obtained at room temperature from all investigated samples exhibit dublet like features (see Fig.1 left side). One of the samples was measured in a broad velocity range (± 12 mm/s) to search for possible occurrence of sextets what would indicate presence of magnetic iron oxides. No traces of any sextet were found. Subsequently, the samples were measured in a narrow velocity range (± 4 mm/s). Low-temperature Mössbauer spectra from all investigated spectra are super-positions of three sextets and one doublet.

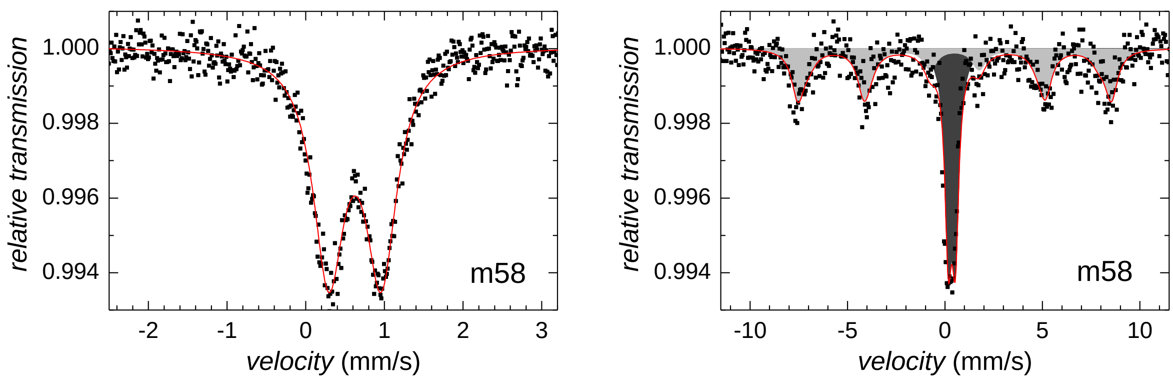

Fig. 1: *Typical Mössbauer spectra of biological tissues measured at room temperature (left side) and at liquid helium temperature (right side).*

Mössbauer parameters of room and low temperature lines for biological tissue are listed in Table 1 and indicate presence of very small particles which exhibit superparamagnetic behaviour. They can be assigned to ferritin-like components. No traces of sextets in room temperature spectra were revealed.

Tab. 1. *Parameters of components (doublets: D1-D3, sextets: S1-S3) derived from Mössbauer spectra of biological tissues measured at room temperature including isomer shift (IS), quadrupole splitting (QS), hyperfine field (B_{hf}) and relative area (A). The errors in the determination of IS, QS, A and B_{hf} are ± 0.04 mm/s, ± 0.02 mm/s, ± 1.5 %, and ± 0.1 T, respectively. Typical values of line widths varied from 0.26 mm/s up to 0.75 mm/s dependence on type of the sub-spectra (doublet and sextet).*

Component	**IS [mm/s]**	**QS [mm/s]**	**A [%]**	**IS [mm/s]**	**QS [mm/s]**	**B_{hf} [T]**	**A [%]**
human brain	room temperature			low temperature			
D1	0.38	0.66	**21**	0.44	0.60		**12**
D2	0.35	0.57	**79**				
S1				0.45	-0.06	50.2	**59**
S2				0.46	-0.13	48.2	**12**
S3				0.45	-0.14	44.1	**17**
human spleen	room temperature			low temperature			
D1	0.37	0.97	**28**	0.41	0.47		**42**
D2	0.45	0.53	**26**				
D3	0.34	0.55	**46**				
S1				0.47	-0.12	50.9	**23**
S2				0.48	-0.12	49.1	**18**
S3				0.45	-0.11	46.6	**17**

However, as it was confirmed by SEM/TEM the size of the ferritin particles [10-11] is so small that there was only a little hope to reveal a presence of some of the minerals mentioned above. The obtained X-ray diffraction pattern of a human spleen exhibited featureless broadened reflections.

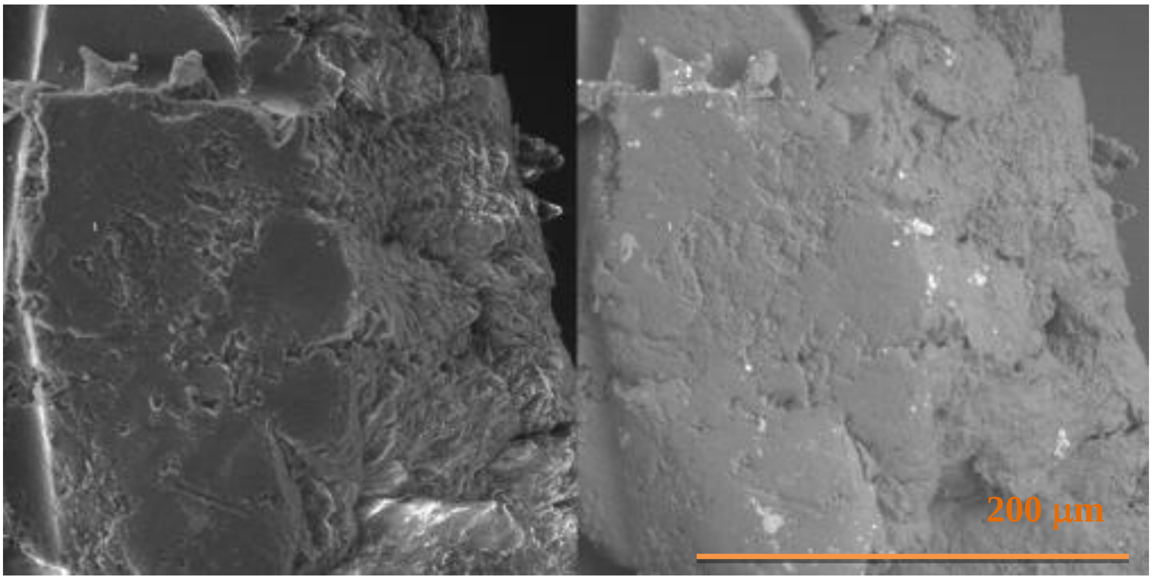

Fig.2: *SEM (left side) and BSE (right side) images of human spleen tissues.*

Figure 2 and Figure 3 show structure of human spleen and human brain tissues, respectively. We can see objects consisting of three arrangements of white, light and dark shadow which confined the composition of different microsphere structure. We assigned the white part on BSE images to the iron in biological tissues.

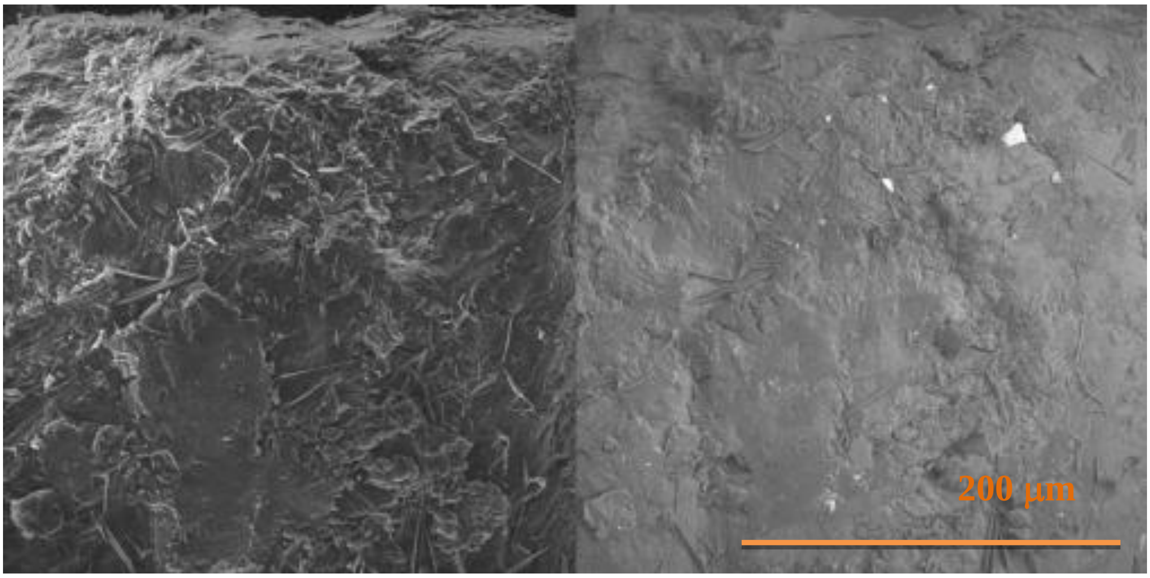

Fig.3: *SEM (left side) and BSE (right side) images of human brain tissues.*

The present results on the Co–Zn ferrites in bulk and nanosize form concern two issues. Extensive investigation was done on two samples of composition $Co_{0.4}Zn_{0.6}Fe_2O_4$ - the 13 nm nanoparticles (prepared at 500˚C) and on bulk samples (ceramics sintered at 1000˚C and subsequently annealed at 500˚C). The first one is the determination of cation distribution that is targeted by a combination of Mössbauer spectroscopy, X-ray and neutron diffraction, while the second one is the spin alignment reflected in the neutron diffraction and magnetization measurements [8]. Typical values of line widths varied from 0.26 mm/s up to 0.38 mm/s dependence on (A) and [B] indicate tetrahedral and octahedral sites of iron, respectively.

Mössbauer spectra measured at room temperature of $Co_{0.4}Zn_{0.6}Fe_2O_4$ consist of doublets and distribution of sextets. This testifies to small particles and/or superparamagnetic phase. Mössbauer spectra measured at helium temperatures are composed different subspectra with dependence on the form of materials. Mössbauer spectra which measured at helium temperatures and external magnetic field for bulk and nanoparticles contain only sextets. All spectra are shown in Fig.4.

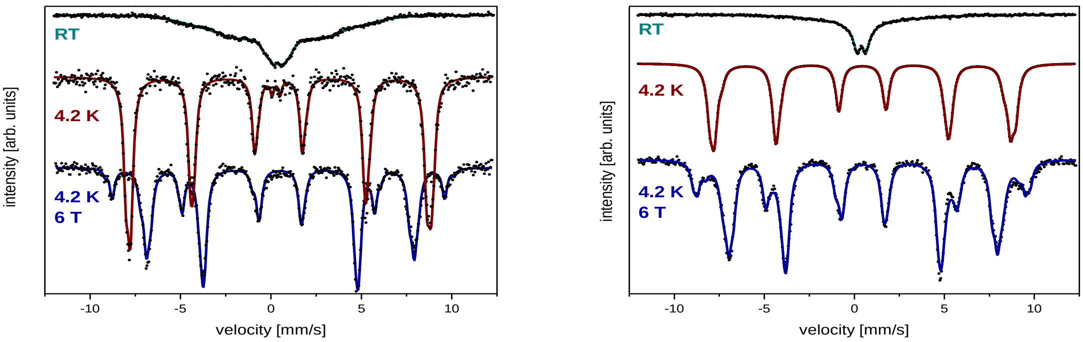

Fig.4: *The typical Mössbauer spectra measured at room and helium temperatures and at 4.2 K with external magnetic field for bulk (left side) and nanoparticles (right side) of* $Co_{0.4}Zn_{0.6}Fe_2O_4$.

The relative area *(A_S)* and ratio from the relative intensity of the second line to the relative intensity of the first line *(I_2/I_1)* of the bulk materials and nano-size form measured at room temperature are listed in Tab. 2.

Tab. 2: *Parameters of components (sextets: S1-S3 and S4-S6 indicate tetrahedral (A) and octahedral [B] sites) derived from Mössbauer spectra of Co–Zn ferrites in bulk and nanosize form at liquid helium temperature including relative area (A_S) and ratio from the relative intensity of the second line to the relative intensity of the first line (I_2/I_1). The errors in the determination of A_S and I_2/I_1 are ± 1.5 %, and ± 0.1 T, respectively.*

	BULK						**NANO**					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
A_S [%]	2	18		13	44	23	10	8	7	29	27	19
I_2/I_1	4	4		3.34	3.34	3.34	2.85	2.85	2.85	2.85	2.85	2.85

4. Conclusions

Nanomaterial is interesting material for biomedical and industrial applications especially in the field of nuclear installations. In this work, we have studied properties of iron-containing nanoparticles in different type of materials; e.g. disks, powders. The methods for preparing of nanomaterials have attracted considerable scientific interest in recent years. These materials are structurally well ordered with very well-defined and exhibit unique physical and chemical properties determined by their practically applications.

Mössbauer spectrometry was chosen as a principal method of investigation. Complex behaviour of magnetic and non-magnetic phases of nanomaterials was identified in the samples by Conversion Electron Mössbauer Spectrometry and transmission technique. Chemical composition was checked by neutron activation analysis and X-ray fluorescence technique. Structural arrangement was studied by scanning electron microscopy with energy dispersive spectrometry. In the presentation, I will discuss the properties of different type of nanomaterials for various applications.

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